Locus	aGvHD Risk	#A	#P	HR Range	p Value Range	DSS Range	DSS Mean
A*	Low	11	389	0.64-1.57	0.2-0.8	1.04-5.66	2.62
	High	4	214	1.78-3.45	<0.001-0.003	1.04-4.3	2.39
B*	Low	4	200	1.28-1.48	0.118-0.538	1.74-2.81	2.53
	High	2	30	3-3.34	<0.001-0.015	1.06-1.06	1.06
Cw*	Low	18	578	0.46-2.88	0.057-0.899	1.52-23.9	15.1
	High	17	600	1.67-6.22	0.001-0.043	1.52-23.86	15.97
DRB1*	Low	25	729	0.47-2.25	0.079-0.985	1.3-13.33	4.91
	High	2	76	2.13-3.19	<0.001-0.003	4.02-10.41	7.22
DQB1*	Low	24	900	0.57-1.58	0.062-0.98	0.24-20.61	10.37
	High	3	114	1.75-2.81	0.002-0.017	1.38-20.61	13.74

combinations are 13.88 and 15.33, respectively. The following table summarizes DSS means and ranges for high and low risk combinations.

(Table 1) #A: # of allele combinations analyzed by Histocheck

#P: # of donor/patient pairs that have all the analyzed combinations

HR: Hazard Ratios of developing severe aGvHD

P values: for the corresponding estimated hazard risk

Conclusion: Our analysis demonstrates that means and ranges of DSS were interchangeable among high and low risk allele combinations within loci A, B, and Cw. In loci DRB1 and DQB1 DSS means were higher in the high risk combinations but the ranges remain overlapping. This analysis does not support selecting donors for HSCT recipients on the basis of low HistoCheck scores.

IMMUNE RECONSTITUTION

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CYTOTOXIC T LYMPHOCYTES (CTL) SPECIFIC FOR CMV, ADENOVIRUS, AND EBV CAN BE GENERATED FROM NAIVE T CELLS FOR ADOPTIVE IMMUNOTHERAPY

Hanley, P.[1], Savoldo, B.[1], Cruz, C.R.[1], Leen, A.M.[1], Rodgers, J.[2], Liu, H.[1], Gee, A.P.[1], Heslop, H.E.[1], Rooney, C.M.[1], Brenner, M.K.[1], Shpall, E.J.[3], Bollard, C.M.[1] [1] Baylor College of Medicine, Houston, TX; [2] Baylor College of Medicine, Houston, TX; [3] M.D. Anderson Cancer Center, Houston, TX

Adoptive immunotherapy with peripheral blood (PB)-derived CMV/Ad/EBV-specific CTL generated from seropositive donors effectively prevents viral disease after Peripheral Blood Stem Cell Transplant (PBSCT), but this option has not been feasible when the donor T-cells are naive. PBSCT from CMV-seronegative (CMVneg) donors to CMV-seropositive (CMVpos) recipients produces a high incidence of CMV infection since donor T-cells are naïve to this virus. Umbilical cord blood (CB) is an important source of stem cells for allotransplant patients lacking human leukocyte antigen (HLA)-matched donors. T-cells in CB grafts are, however, also virus-naïve, leading to higher infections rates with CMV, EBV, adenovirus (Ad) and other viruses. Irrespective of whether the naive T-cells are sourced from CB or CMVneg PB, CTL generation for clinical use from these donors has been unsuccessful. We have now overcome this problem and can routinely generate CMV, Ad and EBV specific CTL from CB and CMV-specific CTLs from seronegative PB **for clinical use**. We used an Ad5f35CMVpp65 vector to transduce CB or PB derived dendritic cells and stimulated virus-specific CTL in the presence of IL-7, IL-12 and IL-15. This was followed by 2 stimulations with autologous EBV-lymphoblastoid cell lines (LCL) transduced with the same vector. CB-derived CTL were predominantly CD8+ (mean 87%;range 81-94) and had significant cytotoxicity against CMVpp65, Adhexon/penton and LCL targets. In addition, we generated CMVpp65, Adhexon/penton and LCL-specific responses from the PB of 4 CMVneg adult donors, which produced a mean of 92 (range 50-126), 163 (range 69-293), and 62 (range 37-86) SFC to CMVpp65 respectively. Neither CB nor CMVneg-derived CTL responded to irrelevant peptides. Of note, the virus-specific T-cells expanded from CB and CMVneg donors derived only from T-cells with a naive phenotype (CD45RA+/CCR7+). Moreover, both CB and CMVneg-derived CTL recognized "unconventional" CMVpp65 epitopes, as identified by overlapping pp65 peptide pools and confirmed by IFN-γ ELISPOT as well as multimer analysis. In HLA-A2+ subjects, naive-derived CTL did not recognize conventional HLA-A2 associated CMV pp65 epitopes such as NLV, suggesting an inherent difference between naïve and memory T-cell responses to CMV. In summary, virus-specific responses T-cell responses can be obtained even from CB and virus-naive adult donors and may allow prevention and treatment of viral disease in the recipients of these allografts.

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DENDRITIC CELL FACILITATE THYMIC RECOVERY AND ENHANCE IMMUNE RECONSTITUTION AFTER HEMATOPOIETIC STEM CELL TRANSPLANT

Langfitt, D., Wallace, N., Dallas, M.H. St Jude Children's Research Hospital, Memphis, TN

After hematopoietic stem cell transplantation (HSCT), the first committed cells to engraft in the thymus are dendritic cells (DCs). The role of thymic DCs in T cell production by ensuring efficient tolerance and selection has been well demonstrated, however the role of thymic DCs in facilitating donor engraftment has not been investigated. Here we show addition of ex-vivo generated DCs accelerates thymic engraftment as well as enhance T cell recovery after HSCT. Control group received 10^3 $lin^-sca\text{-}1^+c\text{-}kit^+$ (LSK) (CD45.2) hematopoietic stem cell progenitors while the DCs group received 10^3 LSK (CD45.2) cells along with 10^3 ex-vivo generated DCs. DCs were generated using bone marrow from GFP^+ CD57/Bl mice (CD45.1/GFP^+) and cultured for 7 days with GMCSF. On the day of HSCT, C57/BL (CD45.1) recipients received lethal radiation at 1000 cGy. At 4 and 7 days after HSCT, thymuses of the DCs group had GFP^+ $CD11c^+$ cells present in the medullary region confirmed by immunohistochemistry and contained 1.8 and 4.2- fold, respectively, higher number of thymocytes compared to control group ($p < 0.05$). Furthermore, thymuses of the DCs group showed a 3.2 and 7.4-fold, respectively, higher number of thymoctyes derived from donor LSK (CD45.2) cells compared to the control group ($p < 0.05$ and $p < 0.007$). Two and four weeks after HSCT, peripheral blood of DCs group contained at least 2.6 and 4.8-fold, respectively, higher numbers of $CD3^+$ cells derived from donor LSK (CD45.2) cells compared to the control group ($p < 0.05$). Here, we demonstrate that ex-vivo generated DCs efficiently migrate and home to the thymic medulla and hasten thymic recovery as demonstrated by the higher number of total thymoctyes. Furthermore, DCs facilitate thymic engraftment as shown by increase number of donor thymoctyes. Lastly, recipients of DCs have earlier generation of de-novo donor derived $CD3^+$ T cells in the peripheral blood. By using the GFP^+ (CD45.1) cells along with donor LSK (CD45.2), we were able to confirm that the facilitation of early thymic recovery was due to the increased engraftment of the donor cells rather than autologous recovery of the host. Thus, this study suggests that DCs committed prior to thymic entry maintains the ability to home to the medullary region and facilitate thymoctye recover and hasten immune reconstitution after HSCT.

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CD40-ACTIVATED B CELLS MIGRATE TOWARDS SECONDARY LYMPHOID ORGANS AND INTERACT DYNAMICALLY WITH T CELLS

Klein-González, N.[1], Balkow, S.[2], Kondo, E.[1], Grabbe, S.[2], Liebig, T.[1], Shimabukuro-Vornhagen, A.[1], Bloch, W.[3], von Bergwelt-Baildon, M.[1] [1] University Hospital of Cologne, Germany; [2] Johannes Gutenberg University, Mainz, Germany; [3] Deutsche Sporthochschule Köln, Germany

B cells have been demonstrated to present antigen to T cells in vivo. CD40-activation dramatically improves antigen presentation by normal and malignant B cells and has therefore been studied as an approach to generate autologous "non-artificial" antigen presenting cells for active immunotherapy. Furthermore, CD40-B cells have recently been shown to expand tumorantigen and viral specific CTL

as well as regulatory T cells and are therefore of great interest for post-transplant immunotherapy. Human B cells when activated via CD40-L/IL-4 can be expanded from small amounts of peripheral blood in 12-14 days. CD40-activated B cells can prime naïve T cells, expand memory T cells and express important surface homing molecules. Nevertheless, it remains unclear whether such cells have the property to attract and interact with T cells in a physiological context and whether CD40-activated B cells migrate to secondary lymphoid organs (SLO) in vivo, a necessary step for an antigen-presenting cell (APC) to induce immunity. To address this question we established a platform to generate murine CD40-activated B cells. At day 14 of culture, these cells are >95% CD19+ and CD80/86/MHCI/MHCIIhi. Murine CD40-activated B cells present a 'homing phenotype'; migrate towards SLO chemokines such as CCL19, CCL21 and CXCL13; and induce T-cell chemotaxis in vitro. Upon CD40L activation, B cells up-regulate CCR7 while down-regulate CXCR5 expression which suggests direction of activated B cells towards the B-zone-T-zone boundary. We compared the homing of GFP+ CD40-activated B cells to resting GFP+ B cells and show for the first time that CD40-activated B cells home to SLO significantly more efficiently than resting B cells. Furthermore, CD40-activated B cells localize in B-cell areas, and a significant fraction move to the B-T boundary close to the T-cell zone. To dissect T-cell-APC interactions on a single cell we analyzed three-dimensional migration in collagen matrix. Interestingly, antigen-loaded CD40-activated B cells differ from immature and mature DC by displaying a rapid migratory pattern undergoing highly dynamic, short-lived (7.5 min) and sequential interactions with cognate T cells. Taken together, these data reveal that CD40-activated B cells can home to secondary lymphoid organs and interact dynamically with T cells thus underlining their potential as cellular adjuvant for cancer immunotherapy.

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MULTIVIRUS-SPECIFIC T CELL IMMUNOTHERAPY TO PREVENT OR TREAT INFECTIONS OF ALLOGENEIC STEM CELL TRANSPLANT RECIPIENTS

Leen, A.M., Gerdemann, U., Christin, A., Ramos, C.A., Heslop, H.E., Brenner, M.K., Rooney, C.M., Vera, J.F. Baylor College of Medicine, The Methodist Hospital, Texas Children's Hospital, Houston, TX

Viral infections cause morbidity and mortality in allogeneic HSCT recipients. We and others have successfully generated and infused adoptive T-cell lines specific for EBV, CMV and Adenovirus using monocytes and EBV-transformed lymphoblastoid cell (EBV-LCL) gene-modified with an adenovector as antigen presenting cells (APCs). We have shown that as few as 2×10^5/kg trivirus-specific cytotoxic T lymphocytes (CTL) proliferated by several logs post-infusion and appeared to prevent and treat even severe viral disease resistant to other available therapies. Despite the encouraging clinical results, broader implementation of this "trispecific" CTL approach is limited by high production costs, complexity of manufacture and the prolonged time (4-6 weeks for EBV-LCL generation, and 6-8 weeks for CTL manufacture – total 10-14 weeks) for preparation.

To overcome these manufacturing limitations we have developed new, GMP-compliant strategies. In place of adenovectors we now use DCs nucleofected with non-viral DNA plasmids encoding LMP2 and BZLF1 (EBV), Hexon and Penton (Adv), pp65 (CMV) and Large T antigen (BK virus) to stimulate T-cells. Furthermore, by pooling nucleofected DCs prior to PBMC stimulation, we can reproducibly generate multivirus-specific CTL lines reactive against all the stimulating antigens, without discernible antigenic competition. Secondly, we have demonstrated that the culture of activated T-cells in the presence of IL-4 (1,000U/ml) and IL-7 (10ng/ml) promotes the survival of both high and low frequency antigen-specific CTL and sustains the breadth of reactivity in our lines. Finally, we have replaced traditional plastic cultureware with a new, gas permeable culture device (G-Rex) which promotes the expansion and survival of large cell numbers in a closed system with minimal technician intervention. By implementing these changes we can now produce multispecific CTL targeting EBV, CMV, Ad, and BK virus at a cost per 10^6 cells that is reduced by >90%, and in just 10 days rather than 10 weeks using an approach that may be extended to additional protective viral antigens. Our approach should be of value for prophylactic and treatment applications for high risk allogeneic HSCT recipients.

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FAST B- AND NK-, RECONSTITUTION EARLY AFTER UNRELATED CORD BLOOD TRANSPLANTATION COMPARED TO (UN)RELATED BONE MARROW TRANSPLANTATION IN CHILDREN

de Pagter, P.J.A.[1], Lindemans, C.[1], Keukens, L.[1], Belitser, S.V.[2], de Weger, R.A.[3], van der Weide, P.[3], Baeten, E.[4], de Gouw, A.P.[4], Bierings, M.[1], Van Baarle, D.[1,4], Bloem, A.[4], Boelens, J.J.[1] [1] University Medical Center Utrecht, Netherlands; [2] Utrecht Institute for Pharmaceutical Sciences (UIPS), University of Utrecht, Netherlands; [3] University Medical Center Utrecht, Netherlands; [4] University Medical Center Utrecht, Netherlands

Early immune reconstitution after allogeneic-haematopoietic stem cell transplantation (HSCT) is important for controlling infectious complications. We compared T-, B- and NK-cell reconstitution during the first 100 days after bone marrow (matched sibling: id-SIB, unrelated bone marrow: u-BM) or cord blood (u-CB) HSCT in a single-center prospective study among pediatric patients.

Between 2006 and 2008, 103 patients were included with a median age of 5 years (range 0-21); 33 recipients received id-SIB, 33 u-BM and 37 u-CB grafts. All patients received myelo-ablative conditioning and in the unrelated donor setting thymoglobulin (10 mg/kg) was added from 4 to 2 days before SCT. The number of CD3+ cells in the graft graft was for uCB 8*10e6 (range 8*10e5 – 4*10e7)/kg, for id-SIB 4*10e7 (range 1 – 9*10e7)/kg and for u-BM 5 *10e7 (range 1*10e7 – 5*10e8)/kg.

Statistical analysis was performed by Kruskal-Wallis rank sum tests and Wilcoxon tests with continuity corrections.

Median follow up was 16 months (range 1-28); overall survival was 73%. Probability of neutrophil at day 60 did not differ among id-SIB u-BM and u-CB recipients (median 23 days, range 3-60 after HSCT, $p = 0.092$). Overall T-cell (CD3+) reconstitution (first 100 days) was similar between the 3 groups, but CD8 + T-cell numbers were higher after id-SIB- and u-BM compared to u-CB HSCT (155 and 309 versus 52 CD8 + T-cells/uL, $p = 0.002$). Interestingly, NK-cell and B-cell numbers were significantly higher in u-CB compared to id-SIB ($p = 0.018$ and $p = 0.0003$, resp.) and u-BM recipients ($p = 0.036$ and $p = 0.0003$, resp.) within the first 100 days. On the long term, in u-CB and id-SIB recipients higher ratios of nave CD4+ and CD8 T-cells were found (u-CB: 62% and 87%, id-SIB: 55% and 57%) compared to u-BM recipients (30% and 17%) at 1 year after HSCT ($p = 0.050$ and $p = 0.029$).

Higher NK- and B-cell numbers early after HSCT indicate a better proliferative capacity of these u-CB stem cells compared to id-SIB and u-BM stem cells. We speculate the observed delayed T-cell reconstitution after u-CB HSCT is caused by a deeper in vivo depletion using the same dose of thymoglobuline in a setting of u-CB grafting where the T-cell dose is 1 log lower than an u-BM graft. Insight in the early immune reconstitution is essential for the future development of immune-mediated therapies and the development of optimal graft specific conditioning regimens to prevent prolonged post-HSCT lymphopenia and the associated viral complications.

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NOVEL H1N1 INFLUENZA A (S-OIV) INFECTION IN RECIPIENTS OF HEMATOPOIETIC STEM CELL TRANSPLANTATION

Strasfeld, L.[1], Espinoza, L.[1], Green, J.[2], Graeme, F.[1], Meyers, G.[1], Ball, E.[2], Castro, J.[2], Mulroney, C.[2], Curtin, P.[2], Maziarz, R.[1], Taplitz, R.[2] [1] Oregon Health and Sciences University, Portland, OR; [2] University of California, San Diego, La Jolla, CA

Background: Respiratory virus infections cause significant morbidity in stem cell transplant patients. Novel H1N1 Influenza A (Swine Origin Influenza A, S-OIV) can result in severe disease in immunocompromised hosts.

Methods: Eleven hematopoietic stem cell transplant (HSCT) patients with influenza A were identified between 4/09 and 7/09. This retrospective analysis summarizes the patient demographics, clinical presentation, influenza testing, treatment, and outcome in these S-OIV-infected HSCT recipients.